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Data sheet

VSIG-3:VISTA [Biotinylated] Inhibitor Screening Assay Kit

Catalog #79782
Size: 96 reactions

BACKGROUND: V-domain Ig Suppressor of T cell activation (VISTA, or B7-H5) is a type I transmembrane protein that functions as an immune checkpoint. V-Set and Immunoglobulin domain containing 3 (VSIG-3, or IGSF11) is a ligand for VISTA which, when bound on activated T cells, inhibits their proliferation and the production of cytokines and chemokines. VSIG-3 expression is elevated in many cancers, especially glioma and melanoma, so the blockade of the VSIG-3/VISTA pathway represents a promising potential cancer immunotherapy strategy.

DESCRIPTION: The *VSIG-3:VISTA[Biotinylated] Inhibitor Screening Assay Kit* is designed for screening and profiling inhibitors of VISTA binding to VSIG-3. This kit comes in a convenient 96-well format, with biotin-labeled VISTA, purified VSIG-3, streptavidin-labeled HRP, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of biotin-labeled VISTA by streptavidin-HRP. Only a few simple steps on a microtiter plate are required for the assay. First, VSIG-3 is coated on a 96-well plate. Next, biotinylated VISTA is incubated with VSIG-3 on the plate. Finally, the plate is treated with streptavidin-HRP followed by addition of an HRP substrate to produce chemiluminescence, which can be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
79491	VSIG-3, Fc-fusion (IgG1), Avi-Tag*	20 µg	-80°C	Avoid multiple freeze/thaw cycles!
71327	B7-H5 Fc fusion, Avi-Tag, Biotin-labeled (VISTA-biotin)*	60 µg	-80°C	
79311	3x Immuno Buffer 1	50 ml	-20°C	
79728	Blocking Buffer 2	50 ml	+4°C	
79742	Streptavidin-HRP	10 µl	+4°C	
79670	ELISA ECL Substrate A	6 ml	Room Temp.	
	ELISA ECL Substrate B	6 ml	Room Temp.	
79699	96-well white microplate	1	+4°C	

**The initial concentrations of VSIG3 and VISTA-biotin are lot specific and will be on the tubes containing the proteins.*

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MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
PBS (Phosphate buffered saline)
Luminometer or microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips

APPLICATIONS: This kit is useful for screening for inhibitors of VISTA binding to VSIG-3 .

STABILITY: Up to 6 months from date of receipt, when stored as recommended.

CONTRAINDICATIONS: Keep final DMSO concentration at or below 1%. Excess DMSO interferes with binding and decreases sensitivity of the assay.

REFERENCES:
Wang, J., *et al. Immunol.* 2019, **156.1**: 74-85.
Xu, W., *et al. Cell. Mol. Immunol.* 2018, **15(5)**: 438-446.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Coating the plate with VSIG-3:

1) Thaw **VSIG-3** on ice. Upon first thaw, briefly spin tube containing **VSIG-3** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining **VSIG-3** in aliquots at -80°C. Note: **VSIG-3** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
2) Dilute **VSIG-3** to 4 µg/ml in PBS.
3) Add 50 µl of diluted **VSIG-3** solution to each well and incubate overnight at 4°C. Leave a couple of wells empty (uncoated), for use with the “Ligand Control” (see below).
4) Dilute **3x Immuno Buffer 1** to **1x Immuno Buffer 1** with water. (Keep a portion of the **3x Immuno Buffer 1** undiluted, for use in Step 1 below).
5) Decant to remove supernatant. Wash the plate three times with 100 µl **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.
6) Block wells by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 1 hour at room temperature with slow shaking. Remove supernatant as described in step 5.

Step 1:

1) Prepare the master mixture: N wells × (10 µl **3x Immuno Buffer 1** + 15 µl distilled water)
2) Add 25 µl of master mixture to each well. Use uncoated wells for the “Ligand Control”.
3) Prepare 10X concentration of test inhibitor in an aqueous-based solution. If the test inhibitor is dissolved in DMSO, make sure the final DMSO concentration in the assay is

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≤1%. For example, to test an inhibitor at 10 µM final concentration, prepare the inhibitor at 1 mM in 100% DMSO. Then dilute 1 mM inhibitor in water to 100 µM, which contains 10% of DMSO. Use 5 µl of the 100 µM inhibitor solution (10% DMSO) in the assay to make a 1% DMSO concentration in the final 50 µl reaction mixture.)

4) For the inhibitor buffer, prepare the same solution as above, but without the test inhibitor (e.g. 10% DMSO in water). The DMSO concentration should be the same as in the 10X inhibitor solution above.

5) Add 5 µl of inhibitor solution to each well designated "Test Inhibitor". For the "Positive Control", "Ligand Control" and "Blank", add 5 µl of the same solution without inhibitor (inhibitor buffer). Incubate at room temperature for one hour with slow shaking.

6) Thaw **VISTA-biotin** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **VISTA-biotin** into single use aliquots. Immediately store remaining undiluted enzyme in aliquots at - 80 °C. Note: ***VISTA-biotin*** *is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

7) Dilute **VISTA-biotin** to 30 ng/µl (approximately 625 nM) in **1x Immuno Buffer 1**. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

8) Add 20 µl of **1x Immuno Buffer 1** to the well designated "Blank".

	Blank	Ligand Control	Positive Control	Test Inhibitor
3x Immuno Buffer 1	10 µl	10 µl	10 µl	10 µl
Distilled water	15 µl	15 µl	15 µl	15 µl
Test Inhibitor	-	-	-	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	-
1x Immuno Buffer 1	20 µl	-	-	-
VISTA-biotin (30 ng/µl)	-	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

9) Initiate reaction by adding 20 µl of diluted **VISTA-biotin** (see Step 1-7) to wells labeled "Positive Control", "Ligand Control" and "Test Inhibitor". Incubate at room temperature for one hour with slow shaking.

10) Decant to remove supernatant. Wash the plate 3 times with 100 µl/well **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.

11) Block wells by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Remove supernatant as in Step 1-10.

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Step 2:

1) Dilute **Streptavidin-HRP** 1000-fold with **Blocking Buffer 2**.
2) Add 100 µl to each well. Incubate for 1 hour at room temperature with slow shaking.
3) Wash plate three times with **1x Immuno Buffer 1**. Tap plate onto clean paper towel to remove liquid.
4) Block wells by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Decant to remove supernatant. Tap plate onto clean paper towels to remove liquid.
5) Just before use, mix on ice 50 µl **ELISA ECL Substrate A** and 50 µl **ELISA ECL Substrate B**, then add 100 µl to each well. Discard any unused chemiluminescent reagent after use.
6) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. “Blank” value is subtracted from all readings.

Reading Chemiluminescence:

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second; delay after plate movement is 100 milliseconds. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the “hole” position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

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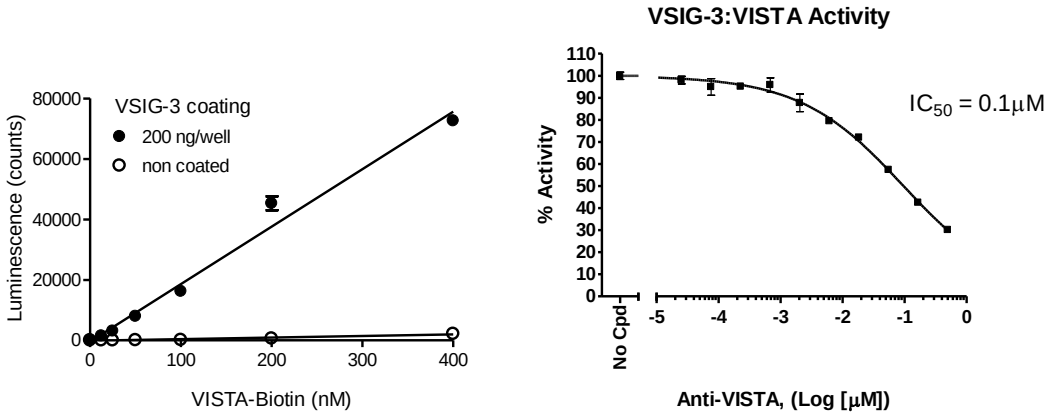
Example of assay results:



VSIG-3:VISTA binding activity, measured using the using the *VSIG-3:VISTA[Biotinylated] Inhibitor Screening Assay Kit*, BPS Bioscience #79782 (left). Inhibition of VSIG-3:VISTA binding using the VISTA/B7-H5/PD-1H Antibody (R&D Systems #AF7126) in the *VSIG-3:VISTA[Biotinylated] Inhibitor Screening Assay Kit* (right). Luminescence was measured using a Bio-Tek fluorescent microplate reader. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

RELATED PRODUCTS:

Product Name	Catalog#	Size
VSIG-3, Fc-fusion (IgG1), Avi-Tag	79491	50 µg
VSIG3, Fc-fusion (IgG1), Avi-Tag, Biotin-Labeled	100044	50 µg
VSIG3, Avi-His-Tag	100349	100 µg
B7-H5 Fc fusion, Avi-Tag, Biotin-labeled (Human)	71327	50 µg
B7-H5 (VISTA), Fc fusion (Human) HiP™	71148	100 µg
B7-H5, Avi-His-Tag	100358	100 µg
B7-H5, Avi-His-Tag, Biotin Labeled, HiP™	100359	50 µg
VSIG8, Fc-fusion (IgG1), Avi-Tag	40743	100 µg

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TROUBLESHOOTING GUIDE

Problem	Possible cause	Solution
Luminescence signal of positive control reaction is weak	VSIG-3 or VISTA-biotin has lost activity	Proteins lose activity upon repeated freeze/thaw cycles. Use fresh VISTA-biotin, (#71327) and fresh VSIG-3 (#79491). Store proteins in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in PBST
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of VISTA-biotin (BPS Bioscience #71327) to create a standard curve